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TITLE: Acute Lung Injury Following Smoke Inhalation: Predictive Value of Sputum Biomarkers and Time Course of Lung Inflammation

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13. ABSTRACT (Maximum 200 Words)

Background: The role of lung inflammatory mediators in the development of lung injury following smoke inhalation is unknown.
Objectives: To evaluate the predictive value and role of inflammatory mediators in acute lung injury following smoke inhalation.
Specific aims: 1) Determine the predictive value of initial inflammatory markers in bronchial secretions of smoke inhalation victims for subsequent lung injury.
2) Measure longitudinal changes in inflammatory mediators in smoke inhalation victims.
Study design: Bronchial secretions from 200-250 intubated patients with smoke inhalation injury will be evaluated for initial and longitudinal changes concentrations of substance P, TNF-α, IL-1, IL-8, and IL-10, as well as cell count and differential every two hours to a maximum of 72 hours. Initial lung inflammation and changes in inflammatory markers will be compared in patients without and without subsequent significant lung injury.
Progress to date: We have enrolled 25 subjects to date in the study, almost all of whom have developed acute lung injury. We have collected detailed clinical outcome data on these subjects and have started to analyze substance P, TNF-α, IL-1, IL-8, and IL-10 concentrations. Determination of the concentrations predictive of the severity of subsequent lung injury await the recruitment and analysis of additional subjects.

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Smoke inhalation, acute lung injury, inflammation, cytokines

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INTRODUCTION

The goal of this research is to identify inflammatory mediators playing key roles in acute lung injury (ALI) following smoke exposure. Our objectives are to determine the value of initial concentrations of these mediators in predicting later development of ALI, and to determine how the mediator concentrations change over time, which may also have predictive value and improve our understanding of the mechanism of smoke injury. We hypothesize that smoke inhalation results in rapid changes (within two hours) in lung inflammatory mediators, initial changes in lung inflammatory mediators are predictive of the extent of subsequent lung injury and changes over time in lung inflammatory mediators will precede clinical findings of acute lung injury. Over the two remaining years of this grant, we will be evaluating initial concentrations and changes over time of inflammatory mediators in pulmonary secretions of approximately 100 ventilated patients with smoke inhalation. The clinical course of these patients will be tracked, including % body surface area burn, days on a ventilator, days in ICU, pulmonary infiltrates, white blood cell count, fever, sputum volume, oxygen requirements, blood oxygenation, and development of ALI.

BODY

The two main specific aims of the study are: 1) Determine the predictive value of initial inflammatory markers in bronchial secretions of smoke inhalation victims for subsequent extent of lung injury; and 2) Measure longitudinal changes in bronchial inflammatory mediators in smoke inhalation victims. The specific aims have been divided into five tasks as shown in the approved Statement of Work timetable (with the task description modified to clarify the meaning of each step).

	Year 1	Year 2	Year 3	Year 4
Recruitment/Enrollment	→→→→→→→→→→→→→→→→			
Tracheobronchal fluid sample collection	→→→→→→→→→→→→→→→→			
Medical outcome data collection	→→→→	→→→→	→→→→	
Sample Analysis	→→→→→→→→→→→→→→→→			
Data analysis/Manuscript preparation	→→→→→→→→→→→→→→→→→→→→			

The major activity of the first year of this research was to obtain Institutional Review Board (IRB) approval from the Army, the University of Arizona, and the Maricopa Integrated Health System (MIHS), which is the parent institution of the Arizona Burn Center where the subjects are enrolled in the study and the bronchial suction material and clinical outcome data collected. This process took much longer than anticipated and therefore required shifting the start of all of the timetable tasks into year 2. We therefore plan to continue subject recruitment and sample collection through year 4.

During this second program year we have begun subject recruitment in earnest. To date, we have recruited 25 subjects, and expect to recruit at least 30 additional subjects in the third program year. Cytokine analysis has been completed and entered into our database for 6 of the subjects, and analysis on additional subjects is ongoing. For subject recruitment, we have found

that obtaining timely consent has limited our ability to enroll more patients in the study. Many patients come to the Arizona Burn Center unaccompanied and in some cases no family members or guardians can be found. For that reason, our anticipated goal of enrolling 80-100 subjects each year will not be met, with a new more reasonable goal of 30-50 subjects per year.

Sample collection for analysis of inflammatory mediators has gone smoothly. Initial samples were collected within 2 hours of exposure in 3 subjects and within 4 hours of exposure in an additional 11 subjects. Following the initial sample collection, subsequent samples were regularly collected in the great majority of subjects. We have experienced difficulty maintaining samples for cell count and differential despite initial success with methanol preservation. We are now trying glycerol preservation and should be able to judge its success within the next few weeks.

We have collected clinical outcome data on 25 subjects and are working to ensure that all key variables are completed on all subjects (Table 1). One unexpected complication has been that essentially all subjects have developed acute lung injury. This has caused us to change our focus from comparison of subjects developing ALI v. those not developing ALI to the outcome variable PaO_2/FIO_2 ratio. Combined with infiltrates on CXR, a PaO_2/FIO_2 ratio less than 300 is diagnostic of ALI and a PaO_2/FIO_2 ratio less than 200 is diagnostic of acute respiratory distress syndrome (ARDS).

Inflammatory mediator concentration measurement has been completed on 6 subjects so far. We spent an extensive amount of time comparing commercially available ELISA kits including the Luminex system which provided multiple mediator measurements on a single sample. However, the results did not correlate significantly among the different ELISA kits. In addition, we found that dilution of samples in order to bring some of the inflammatory mediators into an acceptable range for measurement also greatly increased the variability of our measurements. After considering all factors together, we chose to use R&D QuantiGlo ELISA kits for analysis of inflammatory mediators for IL-1β, IL-8 and TNF-α and R&D high sensitivity kits for IL-10. Substance P measurements have been extremely variable and so far we have not been able to standardize on one assay.

Data analysis is ongoing. For the first 12 subjects, longitudinal analysis of the PaO_2/FIO_2 ratio is illustrated in Figure 1. An example of longitudinal changes in cytokines for one patient is illustrated in Figure 2. In general, IL-10 concentrations have not shown the tremendous increases seen with IL-1β, IL-8 and TNF-α. Evaluation of cytokine concentrations predictive of later development of ALI or ARDS will need to await analysis of samples from additional subjects.

We are beginning to construct regression models to identify independent variables predictive of later injury, with the dependent variable being the lowest PaO_2/FIO_2 ratio measured for each subject. Both age and percent body surface area burned are being evaluated as independent variables given significant or close to significant correlations with lowest PaO_2/FIO_2 ratio measured (Figures 3 and 4). When additional subjects are enrolled in the study, we will change age to a categorical variable given our belief that both very young and very old smoke inhalation victims will have the worst outcomes. We have shown that percent body surface area burned

(range 0-60%) is predictive of the lowest PaO_2/FIO_2 ratio (Table 2). As cytokine analysis is completed on additional subjects, we will add these concentrations into the regression models. After cytokine analysis has been completed on 20-30 total subjects, we will analyze for the predictive value of the current inflammatory mediators studied to see if continued analysis on all of them is warranted.

Table 1. Clinical outcome in smoke inhalation subjects.

Subject	**Age**	**Gender**	**Worst PaO_2/FIO_2**	**Infiltrates**	**ARDS**
1	31	Male	45.60	Yes	Yes
2	17	Male	195.4	Yes	Yes
3	33	Male	91.30	No	No
4	63	Male	197.20	Yes	Yes
5	41	Male	274.50	Yes	No
6	54	Male	120.50	Yes	Yes
7	46	Female	292.40	No	No
8	49	Male	48.70	Yes	Yes
9	48	Male	83.10	Yes	Yes
10	49	Male	108.00	Yes	Yes
11	39	Male	65.20	Yes	Yes
12	24	Male	217.1	Yes	No
13	56	Male	162.3	Yes	Yes
14	21	Male	73.1	Yes	Yes
15	34	Male	71.6	Yes	Yes
16	13	Male	96.5	Yes	Yes
17	67	Male	194.2	Yes	No
18	71	Male	295	Yes	Yes
19	32	Male	99	Yes	Yes
20	34	Male	266.4	Yes	Yes
21	26	Male	86.7	Yes	Yes
24	55	Male	237.0	Yes	Yes
26	70	Male	246.8	Yes	Yes
27	47	Male	275.9	Yes	Yes
30	12	Male	441.1	No	No

Figure 1. Longitudinal analysis of the PaO_2/FIO_2 ratio.

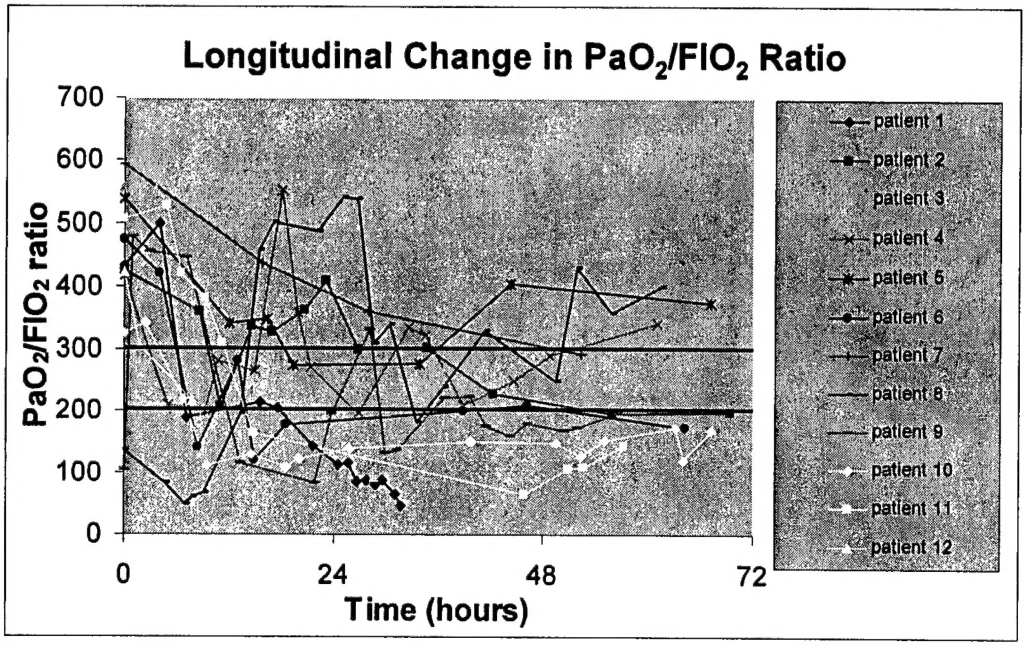


Figure 2. An example of longitudinal changes in cytokines for one subject.

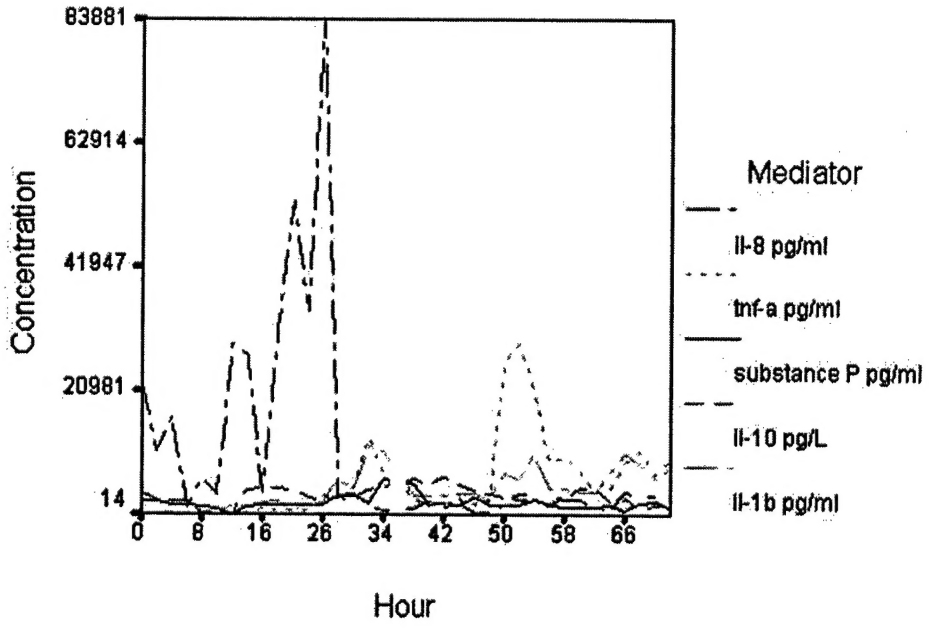
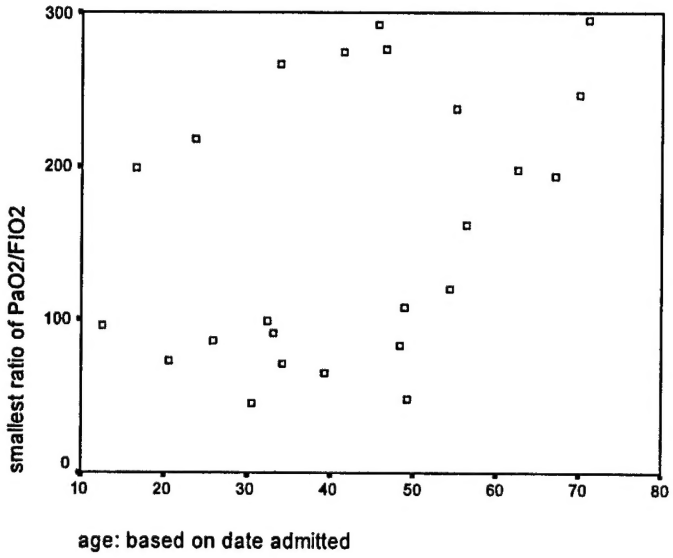
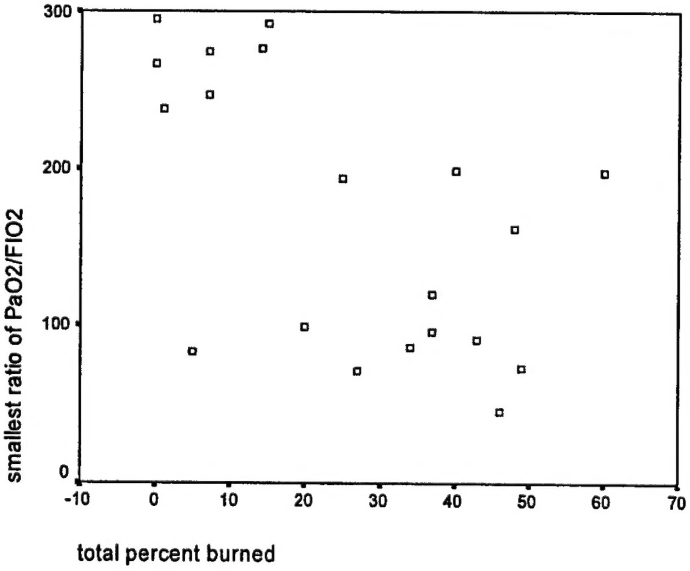


Figure 3. Age and worst PaO_2/FIO_2 ratio



pearsons correlation coefficient=0.3999,p=0.053

Figure 4. Total percent body burned and smallest ratio of PaO_2/FIO_2.



pearsons correlation coefficient= -0.586,p= 0.007

Table 2. Regression coefficients of linear regression model for lowest ratio of PaO_2/FIO_2.

Variable	Coefficent±SE	p-value	95% CI
% body surface burnt	-2.1±.867	0.025	[-3.96,-0.303]
Age	1.8±.928	0.073	[-0.18, 3.73]
Constant	149.3±58.9	0.013	[35.60, 262.9]

Note: As total surface area burnt increases, PaO_2/FIO_2 ratio decreases. As age increases, PaO_2/FIO_2 ratio increases.

KEY RESEARCH ACCOMPLISHMENTS

- We have demonstrated that in our patient population smoke inhalation victims almost uniformly manifest a decline in their PaO_2/FIO_2 ratio to below 300, which coupled with infiltrates on CXR is consistent with development of acute lung injury.
- The lowest PaO_2/FIO_2 ratio is predicted by the total percent body surface area burned, supporting its use as a dependent variable in regression analysis in lieu of ALI v. no ALI.
- We have demonstrated that bronchial suction material can be used for longitudinal analysis of cytokines using commercially available ELISA kits.
- We are in the process of enrolling subjects to begin to evaluate the ability of cytokine concentrations at 2-4 hours following exposure to predictive the later extent of lung injury.

REPORTABLE OUTCOMES

We have presented one poster at a scientific meeting describing our preliminary findings and will present a second within the month. The details are as follows:

1) Department of Defense Peer Reviewed Medical Research Program — April 27, 2004
Military Health Research Forum (poster session), Puerto Rico
"Longitudinal changes in tracheobronchal fluid inflammatory mediators in smoke inhalation victims developing acute respiratory distress syndrome (ARDS)"

2) American Thoracic Society 100th International Conference — May 25, 2005
Orlando, FL
"Longitudinal changes in tracheobronchal suction fluid inflammatory mediators following smoke inhalation"

CONCLUSIONS

Smoke inhalation injury continues to cause significant morbidity and even mortality, as demonstrated by the clinical outcomes of our subjects to date. No diagnostic test or specific

pharmaceutical therapy is available for acute lung injury following smoke exposure. We have shown that longitudinal evaluation of tracheobronchal suctionate from smoke inhalation victims can be analyzed for measurement of inflammatory mediators. If we can show in the coming 1-2 years of this study that certain inflammatory mediators 2-4 hours following smoke exposure are predictive of the later development of ALI, then it will be reasonable to consider evaluation in animal models and if successful, human clinical trials, of pharmacological agents working through antagonism or promotion of the effects of these mediators.

Future directions include the analysis of additional inflammatory mediators and measurement of our present and additional inflammatory mediators in small animal models of smoke exposure. We plan to analyze for concentrations of C5a in our subjects given the availability of C5a receptor antagonist which has shown promise in animal models of neutrophil-mediated injury. The selection of additional inflammatory mediators will also be based in part on the availability of potential therapeutic interventions associated with the selected mediators.

The initial research proposal mentioned linkages with another investigator at the University of Arizona who had submitted a proposal to perform similar work on an animal model to the U.S. Army Medical Research and Materiel Command. Since this other proposal was not funded, we have been investigating the possibility of collaborating with other investigators with experience in animal models of smoke exposure. We had intended to work with Dr. Edgar Kimmel of the Naval Health Research Center/Environmental Health Effects Laboratory (NHRC/EHEL) on development of an animal model that would reflect the findings that we are measuring in humans. This type of collaboration will be essential in the future testing of pharmacologic therapies for smoke inhalation in order to make decisions about whether to carry out human clnical trials. However, Dr. Kimmel's funding was cut and we are continuing to look for collaborators both within the U.S. Armed Forces and with other entities.

REFERENCES

None

APPENDICES (see following pages)

1) Abstract, Department of Defense Peer Reviewed Medical Research Program Military Health Research Forum (poster session), Puerto Rico — April 27, 2004
"Longitudinal changes in tracheobronchal fluid inflammatory mediators in smoke inhalation victims developing acute respiratory distress syndrome (ARDS)"

2) Abstract, American Thoracic Society 100th International Conference Orlando, FL — May 25, 2005
"Longitudinal changes in tracheobronchal suction fluid inflammatory mediators following smoke inhalation"

LONGITUDINAL CHANGES IN TRACHEOBRONCHAL FLUID INFLAMMATIORY MEDIATORS IN SMOKE INHALATION VICTIMS DEVELOPING ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

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BACKGROUND/PURPOSE: Inflammatory mediators play an essential role in the development of human smoke inhalation injury. We hypothesize that initial changes in lung inflammatory mediators are predictive of the extent of subsequent lung injury. **METHODS**: As a first step in investigating this process, mediator concentrations in tracheobronchal secretions of ventilated patients admitted to the Arizona Burn Center following smoke exposure have been collected every two hours over the first 72 hours following admission. Sample supernatants have been analyzed by ELISA. **RESULTS**: Of the first eight subjects enrolled in the study, outcome data is available for seven. All developed acute lung injury (ALI) and five of the seven developed ARDS. Comparing the initial mediator concentration with the peak level in the 4 subjects for whom mediator levels have been analyzed, interleukin (IL)-1, IL-8, and TNF-alpha increased over 100 fold in at least one subject while IL-10 and substance P never increased more than 10 fold. Mean time (hours) to peak concentration for each mediator are as follows: (IL)-1 35 ± 24, IL-8 50 ± 19, and TNF-alpha 53 ± 21. **CONCLUSION**: Longitudinal collection of tracheobronchal suction material provides a means of measuring changes in inflammatory mediators. In the subjects studied so far pro-inflammatory cytokines have increased markedly over time following smoke exposure and the regulatory cytokine IL-10 and substance P have remained low. As additional subjects are recruited, it will be possible to compare longitudinal changes in inflammatory mediators in subjects without marked lung injury and in subjects developing ALI or ARDS. This information will help determine potential inflammatory mediator targets for later animal and potentially clinical trials of pharmacological therapy.

The U.S. Army Medical Research Materiel Command under DAMD17-02-1-0673 supported this work.

[C52] [Poster: E14] Longitudinal Changes in Tracheobronchal Suction Fluid Inflammatory Mediators Following Smoke Inhalation

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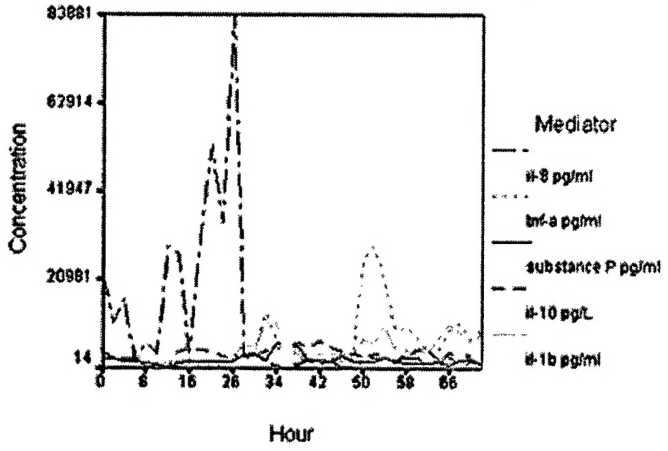
Rationale: The role of inflammatory mediators in the development of human smoke inhalation injury is not well understood. We hypothesize that initial changes in lung inflammatory mediators are predictive of the extent of subsequent lung injury. *Methods*: As a first step in investigating this process, mediator concentrations in tracheobronchal secretions of ventilated patients were collected every two hours over the first 72 hours following smoke inhalation. Sample supernatants were analyzed by ELISA. *Results*: For the first four subjects for which samples have been analyzed, comparing the initial mediator concentration with the peak level gave the following fold increases: interleukin (IL)-1β 70-106; IL-8 6-115; IL-10 1-7; TNF-α 225-2560; and substance P 1-4. The longitudinal changes in one of the subjects enrolled in the study are illustrated in Figure 1. *Conclusions*: Longitudinal collection of tracheobronchal suction material provides a means of measuring changes in inflammatory mediators which will be evaluated for association with development of acute lung injury.

Figure 1. Longitudinal changes in inflammatory mediator concentrations in a single subject



Tuesday, May 25, 2004 8:15 AM

[**] Thematic Poster Session (Abstract Page: A641) Session: 8:15 am-4:15 pm, ENVIRONMENTAL AND OCCUPATIONAL PULMONARY TOXICOLOGY